Endocrine and hyperemic responses to low-intensity aerobic exercise with vascular occlusion

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Abstract

Low-intensity resistance exercise for muscular hypertrophy, combined with vascular occlusion, has previously been shown to promote secretion of plasma growth hormone (GH) because of acute hypoxia in the working muscles. Similarly, a moderate-intensity aerobic exercise (without occlusion) for improvement in endothelial function has been shown to increase blood flow through vessel lumina. Therefore, we investigated whether an enhanced endocrine response and increased blood flow can be achieved simultaneously by combining low-intensity aerobic exercise with occlusion.

To investigate this hypothesis, we examined endocrine and post-exercise hyperemic responses to leg cycle exercise in seven healthy subjects. Exercise was performed at an intensity of about 45% of the maximum heart rate for 20 min, either with or without vascular occlusion applied at the proximal end of both thighs.

Plasma concentrations of GH, noradrenalin, lactate, and nitrite/nitrate, but not insulin-like growth factor 1, increased more after the exercise with occlusion than without occlusion. Maximal blood flow and diameter change in the superficial femoral artery were greater after the exercise with occlusion than that without occlusion.

These results suggest that a low-intensity cycle exercise with vascular occlusion can promote GH secretion through acute hypoxia and accumulation of metabolites, and NO production through enhanced post-exercise hyperemia.

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Introduction

Low-to-moderate intensity resistance exercise with vascular occlusion has been shown to induce increased muscle mass and strength comparable to that seen after conventional heavy resistance training (Takarada *et al.*, 2000b). The physiological mechanism is still not fully understood, but is thought to involve a combination of multiple factors including direct

stimulation of anabolic signalling pathways by mechanical tension developed in muscle during the exercise (Vandenburgh, 1992; Huijing & Jaspers, 2005), additional recruitment of large motor units (Takarada *et al.*, 2000a; Takarada *et al.*, 2000b), acute increase in plasma growth hormone (GH) (Takarada *et al.*, 2000a), and insulin-like growth factor 1 (IGF-1) (Takarada *et al.*, 2000a), increase in muscle growth factors IGF-1 and mechano-growth factor (MGF) (Rennie, Wackerhage, Spangenburg, & Booth, 2004), production of reactive oxygen species (ROS) (Takarada *et al.*, 2000a),and production of nitric oxide (NO) (Kawada & Ishii, 2005).

Acute hypoxia in the region distal to a pneumatic tourniquet, allowing restriction of blood flow during the occlusive resistance exercise, not only stimulates secretion of GH (Viru, Jansson, Viru, & Sundberg, 1998; Takarada *et al.*, 2000a; Takarada *et al.*, 2000b; Pierce, Clark, Ploutz-Snyder, & Kanaley, 2006), but also prolongs and enhances the action of NO (Takehara *et al.*, 2005), an important regulatory molecule in many different tissues, including skeletal muscle (Stamler & Meissner, 2001). In endothelial cells, NO is produced from the amino-acid L-arginine through the action of endothelial nitric oxide synthase (eNOS) (Palmer, Ashton, & Moncada, 1988). The physiological stimulus for endothelial NO production appears to be increased blood flow through the vessel lumen (Pohl, Holtz, Busse, & Bassenge, 1986; Rubanyi, Romero, & Vanhoutte, 1986); shear stress is, in turn, diminished by this NO-mediated vasodilatation. Repeated exposure to such increased blood flow brought about by moderate-intensity aerobic exercise involving the large muscle groups of the legs (e.g., cycling and running), improves NO vasodilator function even in healthy subjects (Clarkson *et al.*, 1999; Kingwell, Sherrard, Jennings, & Dart, 1997).

It is possible that this situation can be reproduced by occlusion, which produces acute hypoxia, and subsequent release of occlusion pressure (viz. reperfusion) when aerobic exercise, even at low intensity, is combined with vascular occlusion. Thus, it is plausible that a low-intensity aerobic exercise combined with vascular occlusion may enhance endocrine responses by hypoxia and activate NO production by increased blood flow.

To investigate this possibility, we examined the acute effects of a low-intensity cycle exercise with vascular occlusion on plasma concentrations of GH, IGF-1, lactate (La), noradrenalin (NA), and nitrite/nitrate (NOx), and as well as post-exercise hyperemia, in healthy subjects. We found that low-intensity cycle exercise, when combined with vascular occlusion, promoted both GH secretion, through hypoxia and accumulation of metabolites, and NO production, through enhanced post-exercise hyperemia.

Methods

Subjects

Seven young, healthy males with no evidence or history of vascular disease were enrolled in the study. Base-line characteristics were as follows: age: 25.8 ± 3.3 yr, height: 170.3 ± 0.8 cm, weight: 67.5 ± 3.6 kg, resting heart rate: (HR) 73.4 ± 2.4 beats/min, systolic blood pressure: 128 ± 4 mmHg, and diastolic blood pressure: 81 ± 6 mmHg.

All subjects were instructed to maintain a nitrite/nitrate-restricted diet (Himeno et al., 2003), and to refrain from medium or heavy physical exercise during 48 h before blood sampling. Subjects were well informed about the purpose of the study as well as the experimental procedures to be utilized, and their written informed consent was obtained. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethical Committee of Faculty of Sport Sciences, Waseda University.

General procedure and exercise protocol

A cycle ergometer (aerobike 900U; Combi Wellness Corp., Tokyo, Japan) was used for leg cycle exercise. HR was continuously monitored by an ear lobe photoelectric pulse sensor connected to the ergometer.

Before starting experimental sessions, we measured baseline diameter of, and blood flow through, the superficial femoral artery (SFA) in the supine position.

Each experimental session started with a 10-min warm-up and muscle-stretching routine. The experimental exercise sessions consisted of 3 types: only occlusion (OO), cycle exercise only (CE); cycle exercise with occlusion (CEO); all 7 subjects participated in all 3 types of exercises, although not in more than 2 on the same day. In OO sessions, subjects remained seated on the cycle ergometer saddle with their feet on the pedals, with vascular occlusion applied by pneumatic tourniquets (width: 90 mm, length: 700 mm), which were tightly fastened around the proximal end of both their thighs, at ~200 mmHg (189 ± 5) mmHg for 20 min. In CE sessions, subjects pedalled the cycle ergometer at an intensity of 40–45% of the individual age-predicted HR_{max} (220-age) (American College of Sports Medicine [ACSM], 2000), with a pedalling rate between 50 and 60 rpm, for 20 min. In CEO sessions, subjects performed 20 min of cycle exercise at 40–45% of HR_{max}, while vascular occlusion was applied to both thighs as in OO sessions.

Following each exercise session, the subject was moved to an examination bed close to the cycle ergometer, and assisted to assume a supine position on the bed for the measurement of diameter of, and blood flow through, the SFA, as well as for taking blood samples. When a suitable B-mode image of the SFA was found (see below), the tourniquets attached to the proximal end of both thighs were rapidly deflated, and B-mode images acquired; blood samples were then also taken as soon as

possible after exercise as well as at set intervals.

Measurements and analyses of diameter and blood flow

Blood flow and diameter of the SFA were measured by means of a high-resolution ultrasound machine, using a 7.5 MHz multi-frequency linear array probe (SDU-2200; Shimadzu Corp., Tokyo, Japan), by the same operator for all experiments.

Ultrasonic parameters were set to adequately optimize longitudinal, B-mode images (length: 4 cm) of the lumen/arterial wall interface. A pulsed-Doppler signal was corrected for angle (70°) to the vessel, with the range gate (4 mm) in the centre of SFA. Once set, these parameters remained constant for all measurements.

First, the SFA was identified and imaged, using two-dimensional B-mode imaging, within the region distal to the tourniquet that was fastened around the right thigh, and just tangent to the surface of the skin. The probe was held in a constant position for each subject; the precise distance of the probe from the upper part of patella was measured and marked on the skin to allow standardized measurement for subsequent sessions.

Prior to, and again after each exercise session, 3 ECG-gated end-diastolic B-mode images were continuously acquired for a duration of 8 min and stored on a digital videotape (GV-D900; Sony Corp., Tokyo, Japan) connected with the ultrasound machine.

Post-exercise analysis of SFA vessel diameter and blood flow was performed by a single investigator, who was blinded to the inter-observer variability of the custom-designed edge-detection software (FMDscope; MEDIA CROSS Corp., LTD, Tokyo, Japan). Diameters were always calculated from the lumen/arterial wall interface at the distal and proximal vessel wall. Intra-class correlation coefficient (ICC) was 0.84. All SFA diameter values were expressed as a percentage of the end-diastolic baseline diameter during the resting state.

Blood flow per minute was calculated by multiplying the velocity-time integral of the Doppler flow signal (corrected for angle of 70°) by HR and the cross-sectional area of the vessel (πr^2).

Blood sampling and Biochemical analyses

Venous blood samples (10 ml for each point of measurement) were obtained from subjects through an indwelling cannula in a superficial vein of either the left or the right forearm. A resting blood sample was obtained after a 15–20 min equilibration period; exercise sessions started 5–10 min after this resting blood sample was drawn. Immediately after exercise sessions, blood samples were obtained (0 min), and again at 5, 10, 15, 30, 45 and 60 min after the session. All blood sampling was conducted during the same time of day to reduce the

effects of diurnal variation on the hormonal concentrations within a given subject. All blood samples were processed and stored at -20°C until analysis.

Plasma concentrations of La, NA, GH, IGF-1, and NOx were measured using spectrophotometry for a lactate dehydrogenase-coupled enzymatic system (Asanuma *et al.*, 1985), HPLC (Yoshimura, Komori, Nakanishi, & Takanashi, 1993), an Access ultrasensitive human GH assay (UniCel DXI 800; Beckman Coulter Inc., Chaska, MN, USA), an immunoradiometric assay kit (SomatomedinCII Bayer; Bayer Corp. Medical Ltd., Tokyo, Japan), and a modified HPLC assay, as described in a previous study under the automated system TCI-NOX1000 (Tokyo Kasei Kogyo Corp., Tokyo, Japan), respectively (Green *et al.*, 1982).

Statistical analysis

All variables were expressed as means ± SE (n = 7). Data for plasma concentrations, blood flow, and percentage of diameter change were analyzed by a two-factorial ANOVA, with a repeated-measured design [Session (OO, CE, CEO) × time of taking blood samples (rest, 0, 5, 10, 15, 30, 45, and 60 min). Similarly, for maximal blood flow through, and maximal diameter of, the SFA, ANOVA with repeated measures (within-subject factor: Session) was used. Where necessary, the Greenhouse-Geisser correction was applied to adjust the degrees of freedom for sphericity violations, using the correction coefficient epsilon. For post-hoc t-tests, a Tukey's method for multiple comparisons was used. Effects were considered significant if $p < 0.05$. For intra-rater reliability, ICC was calculated. All statistical analyses were performed with SPSS 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

Average intensity of cycling achieved by subjects was 71.1 ± 3.0 W. The increase in heart rate, from 6 min after the start of a session until its end, was greater during CEO than during OO or CE ($p < 0.05$; ANOVA, **Fig. 1**). The average heart rate during OO, CE, and CEO was 82.7 ± 0.9, 92.8 ± 1.4, and 116.7 ± 3.6, respectively, which, during COE, was approximately equal to the value of 60% of HR_{max}.

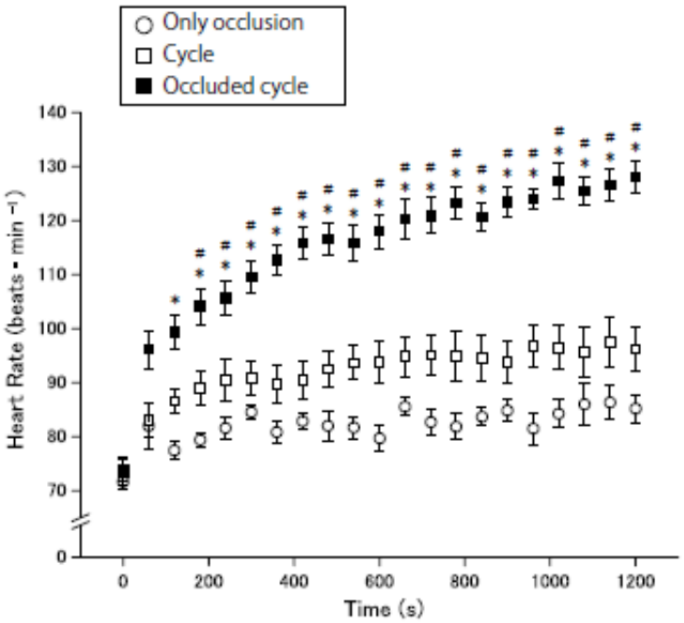

Figure 1. Changes in heart rate during cycle exercises with (filled square, CEO) and without occlusion (open square, CE) and only occlusion without any exercise stimulus (open circle, OO) for 20 min. Data are shown as plots of means ± SE. Each * and # denotes statistically significant differences between CEO and OO, and between CEO and CE, respectively ($p < 0.05$, ANOVA with a Tukey's method for multiple comparison).

Plasma concentrations of La, NA, and GH

Figure 2 shows changes in plasma concentrations of La, NA, and GH, as measured before and after the three experimental exercise sessions. The concentration of all three of these increased rapidly and markedly after CEO, whereas they remained essentially unaltered after OO and CE. A two-factorial ANOVA with repeated-measures design revealed that changes in plasma concentrations of La, NA, and GH were significantly different between the 3 exercise sessions [La: $F(2, 12) = 6.57$, $p < 0.05$, NA: $F(2, 12) = 7.27$, $p < 0.01$, GH: $F(2, 12) = 12.48$, $p < 0.01$; main effect by session, for factors: session (3) × time of blood collection (8)]. These changes were attained from 0 min to 5 min after CEO, after which they gradually returned to their resting levels; the time-course involved in these changes, particularly for La and GH, appeared to be similar. In particular, statistically significant differences between CEO, on the one hand, and OO and CE, on the other, were observed at 0, and 10 min after session for La, 0 min after session for NA, and 0, 5, and 10 min after session for GH ($p < 0.05$).

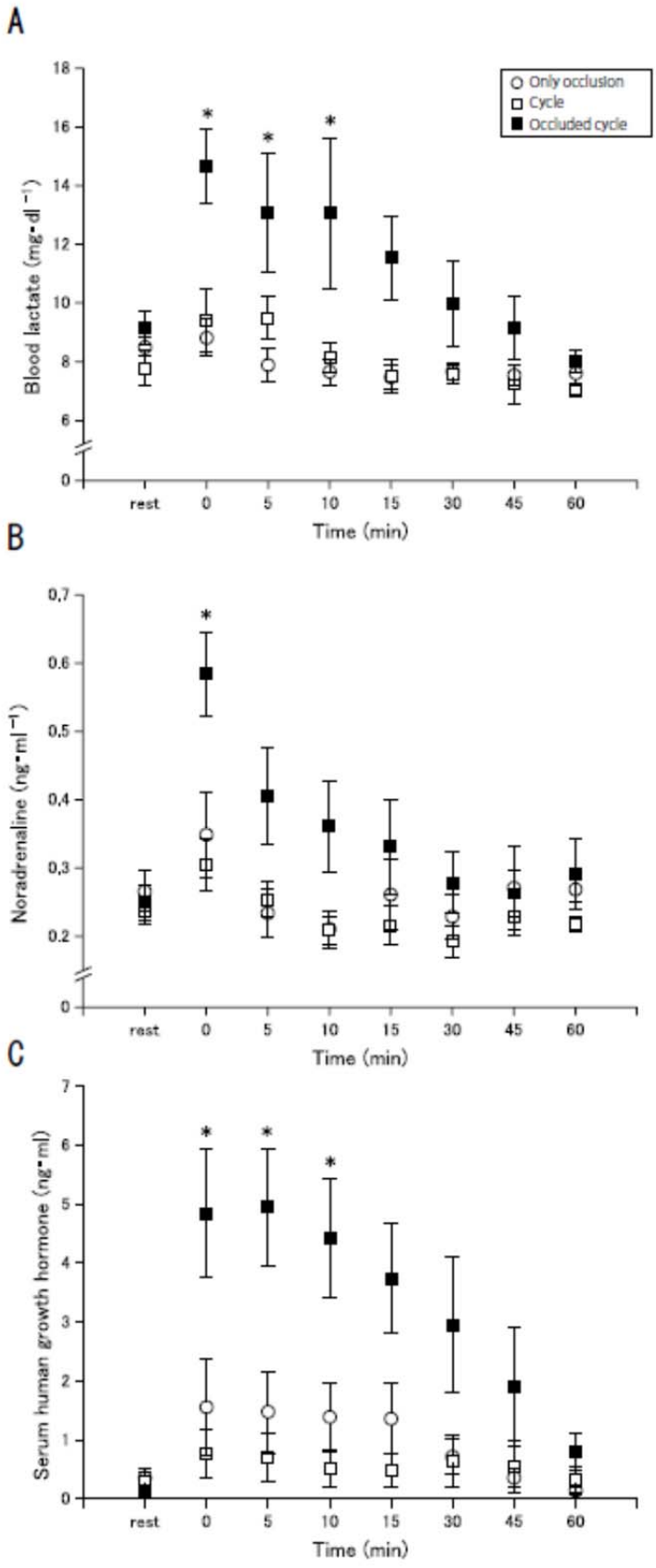

Figure 2. Changes in plasma concentrations of lactate (A), noradrenalin (B), and growth hormone (C) after cycle exercises with (CEO, filled square) and without occlusion (CE, open square), and only occlusion without any exercise stimulus (OO, open circle). Means ± SE were plotted. Rest, before; 0, immediately after. * $p < 0.05$, ANOVA with a Tukey's method for multiple comparison.

Plasma concentrations of IGF-I and NOx

Figure 3A shows changes in plasma concentration of IGF-I measured before and after the three experimental sessions: no statistically significant differences in plasma concentrations of IGF-I were observed between sessions [IGF-I: F (1.13, 6.83) = 0.81, p = 0.466; main effect by session, for factors: session (3) × time of blood collection (8)].

Figure 3B shows changes in plasma concentration of NOx measured before and after the three exercise sessions. Here, statistically significant differences in plasma concentration of NOx were noted between the 3 exercise sessions [F (2, 12) = 7.13, $p < 0.01$; main effect by session, for factors: session (3) × time of blood collection (8)], while statistically significant differences between CEO, on the one hand, and OO and CE, on the other, were observed only at 0 min after session ($p < 0.01$).

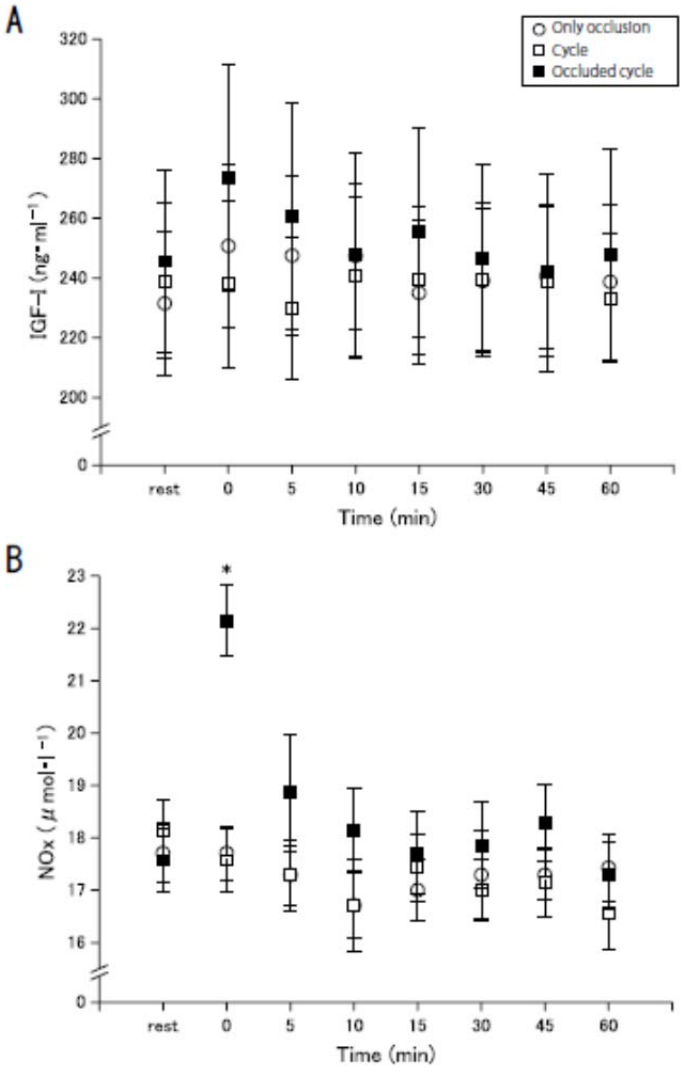

Figure 3. Changes in plasma concentrations of insulin-like growth factor (IGF-1, A) and nitrite/nitrate (NOx, B) after cycle exercises with (CEO, filled square) and without occlusion (CE, open square), and only occlusion without any exercise stimulus (OO, open circle) were plotted as means ± SE. Rest, before; 0, immediately after session. * $p < 0.01$, ANOVA with a Tukey's method for multiple comparison.

Blood flow and diameter change

There were statistically significant differences in both blood flow and the percentage of change in diameter of the SFA, compared to base-line values, between the 3 exercise sessions [blood flow: F (1.10, 5.52) = 95.08, p < 0.01, diameter change: F (1.12, 6.72) = 17.82, p < 0.01; main effect by session, for factors: session (3) × time of measurement (21), **Fig. 4**].

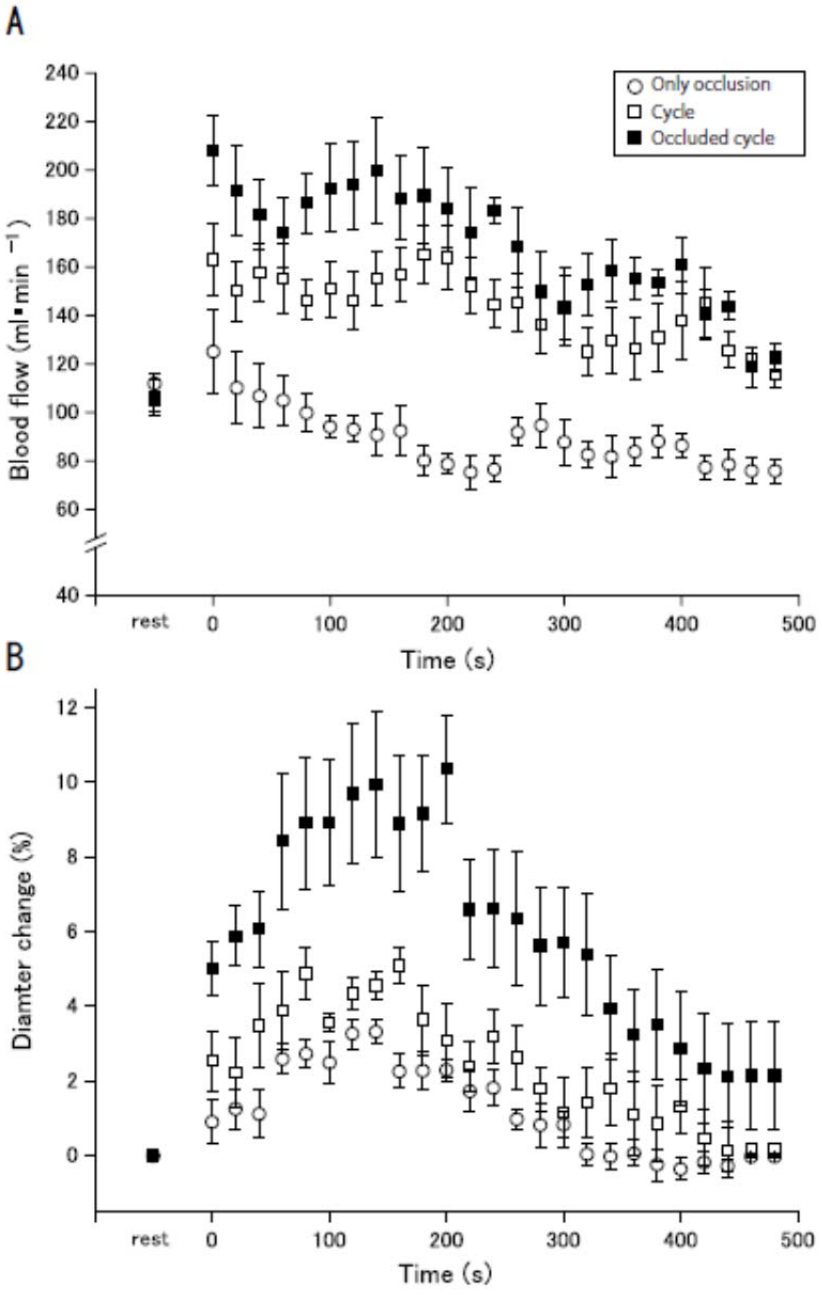

Figure 4. Blood flow (A) and diameter change, calculated as a percentage of the resting state diameter of superficial femoral artery (B) before and after cycle exercises with (CEO, filled square) and without (CE, open square) occlusion, and only occlusion without any exercise stimulus (OO, open circle). Means ± SE were plotted.

Moreover, statistically significant differences between the three types of exercise were observed for maximal blood flow as well as for maximal diameter change [blood flow (Fig. 5): F (2, 18) = 14.19, $p < 0.01$, diameter change: F (2, 18) = 18.87, $p < 0.01$, one-way ANOVA with repeated measures within subject factor: session]. Also, both maximal blood flow and maximal diameter change were significantly greater after CEO than after OO and CE ($p < 0.01$, **Fig.5**).

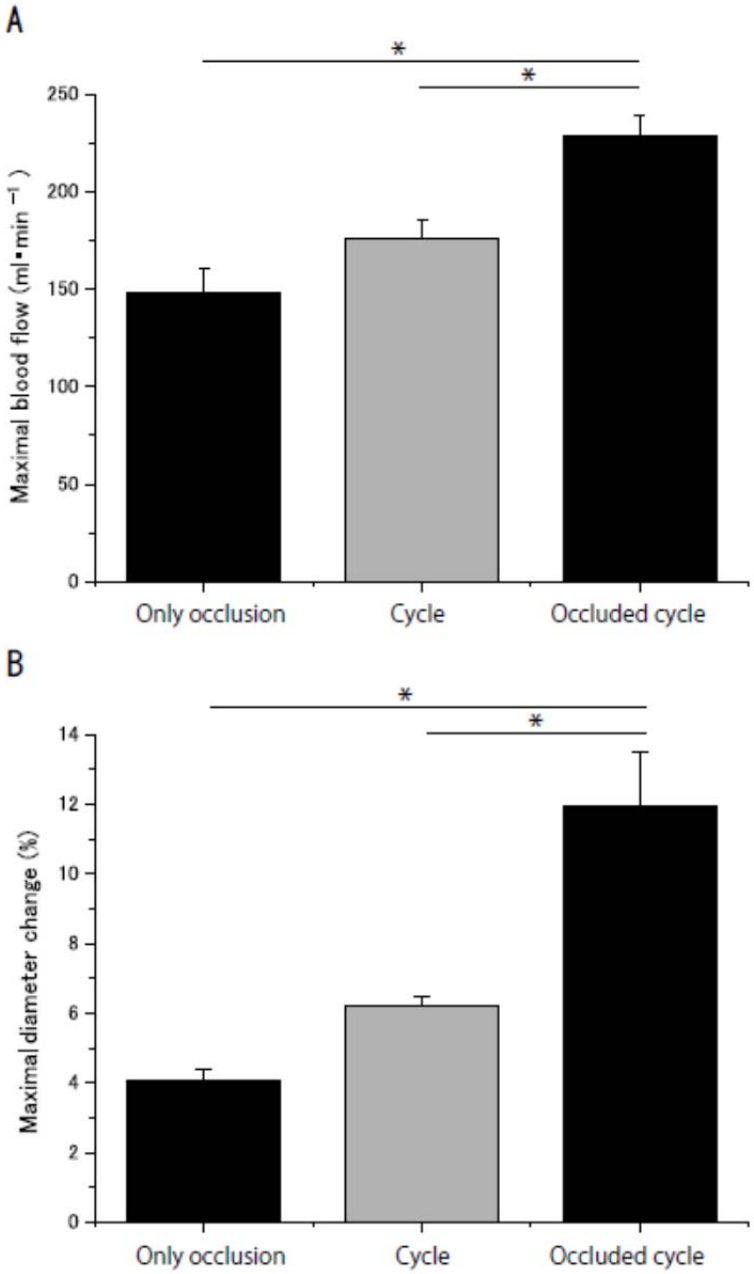

Figure 5. Maximal blood flow through (A) and maximal diameter change, calculated as a percentage of the resting state diameter of the superficial femoral artery (B) after cycle exercises with (CEO, black bars) and without (CE, grey bars) occlusion, and only occlusion without any exercise stimulus (OO, white bars). Data are represented as means ± SE. * $p < 0.01$, ANOVA with a Tukey's method for multiple comparison.

DISCUSSION

The present study showed that a low-intensity cycle exercise combined with vascular occlusion enhanced both endocrine and post-exercise hyperemic responses. The increase in plasma GH concentration was greater in magnitude than that reported to occur after typical strength exercises for large muscle groups of the whole body [5 repetition maximal (RM) with 1 or 3 min rest intervals or 10 RM with a 3 min rest interval] (Kraemer *et al.*, 1990). Blood flow through the SFA after the occlusive low-intensity cycle exercise reached a level comparable to that observed in a preliminary study of moderate-intensity (65% of HR_{max}) cycle exercise *without* occlusion. This resulted in a marked increase in the change in diameter, with elevated NO production probably accounting for the improvement in vasodilatation.

The peak plasma concentration of La after CEO was nearly double the concentration after CE. This increase in La concentration was presumably caused by both local hypoxia, which makes metabolism more anaerobic, and the suppression of La clearance within working muscles under restriction of blood flow. Such lactic acidosis has been shown to stimulate sympathetic nerve activity through a chemo-receptive reflex mediated by intramuscular metabo-receptors and group III and IV afferent nerve fibres (Victor & Seals, 1992), which may be, at least partially, related to the markedly increased HR observed during CEO in our study.

The same chemo-reception pathway has been shown to play an important role in the regulation of hypophyseal secretion of GH (Gosselink *et al.*, 1998). Again, in our study, the finding that changes in NA and GH concentrations were apparently in phase with that of La concentration, supports this concept (Fig. 2); these results also agree substantially with previous results reported by Takarada et al. (2000a). However, the increase in plasma GH concentration observed in the present study was lower in magnitude than that reported in the study by Takarada et al. (2000a); one explanation for this lower GH response may be a difference between the studies in the level of intramuscular hypoxia attained (Ha¨kkinen & Pakarinen, 1993). In fact, the peak lactate level in the present study reached 14.6 mg/dl, whereas in the previous study it reached 44.0 mg/dl (Takarada *et al.*, 2000a).

Plasma concentration of IGF-1 has been shown to behave in a broadly similar manner, even though the changes in concentration are much smaller than is observed for GH. IGF-1 increases protein synthesis during resistance training and enhances muscle hypertrophy (Kahn, Hryb, Nakhla, Romas, & Rosner, 2002); however, the peak concentration of IGF-1 may not be reached until 16–28 h after the stimulated GH release (Chandler, Byrne, Patterson, & Ivy, 1994). Thus, the delayed secretion of IGF-1 may explain the smaller increases in

IGF-1 during the observation period.

The precise role for GH in exercise-induced muscular hypertrophy is currently in contention (Rennie, 2003; Weber, 2002); however, it is incontrovertible that conventional heavy resistance exercise for muscular hypertrophy increases GH concentration for 30 min post-exercise (Kraemer *et al.*, 1990), and that such a marked increase in GH concentration can also be observed after a low-intensity resistance exercise with vascular occlusion (Viru *et al.*, 1998; Takarada *et al.*, 2000a; Pierce *et al.*, 2006). Though Viru et al. (1998) and Pierce et al. (2006) examined GH production in response to ischemic resistance exercise, they did not measure NO production, nor did they observe post-exercise hyperemia.

McCall, Byrnes, Fleck, Dickinson, & Kraemer (1999) have demonstrated that acute changes in plasma concentration of GH after exercise correlate positively with the extent of muscular hypertrophy that occurs after the period of exercise training. Moreover, a low-intensity leg resistance exercise with vascular occlusion has been shown to increase circulating GH concentration, resulting in an increased synthesis of skeletal muscle protein (Fry *et al.*, 2010). Circulating GH, in turn, stimulates synthesis and secretion of IGF-1 within the muscle, which then facilitates growth of the muscle (DeVol, Rotwein, Sadow, Novakofski, & Bechtel, 1990; Isgaard, Nilsson, Vikman, & Isaksson, 1989; Turner, Rotwein, Novakofski, & Bechtel, 1988).

The enhancement of post-exercise hyperemia by low-intensity cycle exercise with vascular occlusion could be directly related to the occlusion and reperfusion involved in the occlusive exercise, leading to greater change in the diameter of the SFA. Acute change in fluid mechanical forces imposed by the circulating blood has been shown to enhance endothelial NOS activity, the magnitude of which increases with the rate of flow (Rotto, Massey, Burton, & Kaufman, 1998). This would induce the rapid release of NO from the vascular endothelium; moreover, hypoxia and reperfusion also activate production of NO (Malinski, Bailey, Zhang, & Chopp, 1994; Takehara, Kanno, Yoshioka, Inoue, & Utsumi, 1995). Thus, the increase in NOx plasma concentrations after CEO occurred as were to be expected.

Vascular occlusion during the low-intensity cycle exercise may cause a pooling of blood in the capacitance vessels in the distal portion of the leg, with a concurrent decrease in blood inflow through the arteries. However, it is very likely that venous outflow and retrograde blood flow, particularly in the femoral artery, would be promoted by the "pumping" actions of stronger muscle contractions (Bonde-Petersen, Henriksson, & Nielsen, 1975; Mitchell, Payne, Saltin, & Schibye, 1980; Walloe & Wesche, 1988). Stronger muscle contractions would occur during occlusive exercise, because the hypoxic and acidic intramuscular

environment brought about by this type of exercise induces incorporation of additional larger motor units, consisting of fast, type II fibres (Takarada *et al.*, 2000b). Such a retrograde blood flow has also been shown to play a crucial role in the action of an oscillatory antegrade/retrograde flow pattern on shear-mediated release of NO from the endothelium (Green et al., 2005), which may be related, at least partially, to the increase in NO production after CEO in the present study.

In skeletal muscle, NO has been suggested to be involved in the expression of cyclooxygenase 2 (COX-2) (Soltow, Betters, Sellman, Lira, Long, & Criswell, 2006). The COX enzymes catalyze production of prostaglandins from arachidonic acid; one of these, prostaglandin $F_{2á}$, may regulate skeletal muscle hypertrophy by activating both cell fusion and protein synthesis (Horsley & Pavlath, 2003). The increased levels of prostaglandin and arachidonic acid in skeletal muscle of cats after isometric muscle contraction in the presence of ischemia have been shown to be higher than for those without ischemia (Rotto, Massey, Burton, & Kaufman, 1989; Symons, Theodossy, Longhurst, & Stebbins, 1991). These findings suggest that NO plays some role in the adaptation of muscle to exercise and occlusive stimuli.

The results presented above imply that a low-intensity cycle exercise with vascular occlusion brings about acute responses similar to those observed in low-intensity *resistance* exercise aimed at muscular hypertrophy, and a moderate-intensity aerobic exercise aimed at improvement in vasodilatation. In particular, the exercise described here increased plasma GH through hypoxia and accumulation of metabolites, as well as post-exercise hyperemia with increases in NO production. It would be reasonable to deduce that long-term exercise training based on the described methodology would increase muscular strength, with concomitant muscle hypertrophy, and improve vasodilatation. This type of exercise would also potentially be useful for subjects who are not able to exercise at greater than moderate exercise intensity.

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